PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 45/05, 37/00, C12N 15/19 C12N 15/24, 15/09, C07K 13/00	A1	**(11) International Publication Number:** WO 93/05814 **(43) International Publication Date:** 1 April 1993 (01.04.93)

(21) International Application Number: PCT/US92/07994

(22) International Filing Date: 21 September 1992 (21.09.92)

(30) Priority data:
763,443 20 September 1991 (20.09.91) US

(71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 02114 (US).

(72) Inventors: STAMENKOVIC, Ivan ; 59 Babcock Street, Brookline, MA 02146 (US). SGROI, Dennis, C. ; 93 Emerson Garden, Lexington, MA 02173 (US).

(74) Agent: CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110-2804 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).

Published
With international search report.

(54) Title: COMPETITIVE INHIBITION OF T CELL-B CELL INTERACTIONS

(57) Abstract

A method of inhibiting, in a biological sample the binding of a CD22β-bearing B cell to a second cell bearing a CD22β-specific ligand, by contacting the sample with a substance which binds to said CD22β-specific ligand to competitively inhibit the binding of the B cell to the second cell. This second cell may, for example, be a T cell or B cell. In addition, the invention features a method of competitively inhibiting the binding of B cells to T cells in a human patient, thereby preventing activation of both T and B cells, by administering an inhibiting amount of a composition including a soluble protein comprising a portion of CD22β capable of binding to a CD22β-specific ligand on a T cell, and a pharmaceutically acceptable carrier.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**FI**	Finland	**MN**	Mongolia
AU	Australia	**FR**	France	**MR**	Mauritania
BB	Barbados	**GA**	Gabon	**MW**	Malawi
BE	Belgium	**GB**	United Kingdom	**NL**	Netherlands
BF	Burkina Faso	**GN**	Guinea	**NO**	Norway
BG	Bulgaria	**GR**	Greece	**NZ**	New Zealand
BJ	Benin	**HU**	Hungary	**PL**	Poland
BR	Brazil	**IE**	Ireland	**PT**	Portugal
CA	Canada	**IT**	Italy	**RO**	Romania
CF	Central African Republic	**JP**	Japan	**RU**	Russian Federation
CG	Congo	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CH	Switzerland			**SE**	Sweden
CI	Côte d'Ivoire	**KR**	Republic of Korea	**SK**	Slovak Republic
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CS	Czechoslovakia	**LK**	Sri Lanka	**SU**	Soviet Union
CZ	Czech Republic	**LU**	Luxembourg	**TD**	Chad
DE	Germany	**MC**	Monaco	**TG**	Togo
DK	Denmark	**MG**	Madagascar	**UA**	Ukraine
ES	Spain	**ML**	Mali	**US**	United States of America

COMPETITIVE INHIBITION OF T CELL-B CELL INTERACTIONS

Background of the Invention

This invention relates to T cell-B cell interactions.

To differentiate into antibody producing cells, B lymphocytes must interact with helper T cells. Helper T cells are thought to stimulate B cells indirectly, by secreting B lymphotropic cytokines (see, e.g., Dutton et al., Prog. in Immunol., 1:355-68 (1971); and Kishimoto and Hirano, Annu. Rev. Immunol., 6:485-512 (1988)), and directly through physical cell-cell contact (see, e.g., Kupfer et al., Proc. Natl. Acad. Sci., USA, 63:6060-83 (1986); and Noelle et al., J. Immunol., 143:1807-14 (1989)). While the role of cytokines in T cell-dependent B cell activation has been investigated (Kishimoto, Annu. Rev. Immunol., 3:133-67 (1985); Kishimoto and Hirano, supra), the molecular nature and physiologic effects of physical association between B lymphocytes and helper T cells are not yet fully understood.

Several receptor-ligand pairs have been proposed to participate in T cell adhesion to B cells, including CD2-LFA3, CD4-MHC class II, T cell receptor-antigen/MHC class II and LFA1-ICAM-1/ICAM-2 (Springer et al., Annu. Rev. Immunol., 5:223-52 (1987); Springer, Nature, 346:425-33 (1990)). However, most of these molecules are thought to play a non-specific accessory adhesion role and are involved in T cell interaction with a variety of antigen presenting cells (Springer, supra).

Mature B lymphocytes express a lineage-specific cell surface receptor, CD22, a 130/140kD heterodimer (Dorken et al., J. Immunol., 136:4470-79 (1986)), composed of two independently expressed polypeptide chains (Boue and Lebien, J. Immunol., 140:192-99 (1988)). Recent isolation of CD22 cDNA clones has revealed that

both CD22 polypeptides are members of the immunoglobulin superfamily of integral membrane proteins (Stamenkovic and Seed, Nature, 344:74-77 (1990); Wilson et al., J. Exp. Med., 173:137-46 (1991)). The smaller form, CD22α, has an extracellular region composed of 5 Ig-like domains (Stamenkovic and Seed, supra) while the larger form, CD22β, has two additional Ig-like domains (Wilson et al., supra). Both polypeptide chains are highly related to myelin associated glycoprotein (MAG), neural cell adhesion molecule (N-CAM), and the vascular adhesion molecule V-CAM/InCAm-110 (Stamenkovic and Seed, supra, Wilson et al., supra), consistent with a role in cell-cell adhesion.

Initial studies on the function of CD22 revealed that CD22α mediates adhesion to erythrocytes and monocytes (Stamenkovic and Seed, supra) while CD22β participates in B cell-B cell interactions (Wilson et al., supra). Cell surface expression of CD22 on B lymphocytes coincides with the capacity to respond to antigen (Pezzutto et al., J. Immunol., 140:1791-95 (1988)), and the expression pattern of CD22 is reminiscent of that of IgM (Dorken et al., supra).

Recently, CD45 molecules have been shown to display phosphotyrosine phosphatase activity (Tonks et al., Biochemistry, 27:8695-701 (1988); Hunter, Cell, 58:1013-16 (1989)) and were proposed to regulate signal transduction in lymphocytes by enhancing or blocking cell activation induced through T or B cell surface antigens. The regulatory function is believed to result from interaction between intracellular portions of CD45 and various lymphocyte cell surface molecules. Depending on the cell surface molecules with which CD45 interacts, the resulting signals may be stimulatory or inhibitory (Clark and Ledbetter, Today, 10:225-28 (1989)). Cross-linking of CD45 with CD3 or CD2 inhibits the ability of anti-CD3

and anti-CD2 mAb to increase intracellular calcium fluxing and stimulate T cell activation. Conversely, cross-linking of CD45 with CD4 greatly augments the calcium fluxing produced upon cross-linking CD4 alone (Ledbetter et al., Proc. Natl. Acad. Sci. USA, 85:8628-32 (1988)), suggesting that CD45 may regulate early activation events in T cells. Although it is widely believed that CD45 isoforms participate in cell-cell interactions (Hunter, supra), the corresponding ligands on adjacent cells have remained elusive.

When cells of the immune system encounter an antigen, a humoral or a cellular immune response, or both, may ensue. Humoral immunity is mediated by B cells, whereas cellular immunity is mediated by T cells. The interaction of antigen on an antigen-presenting cell with a helper T cell is a critical first step leading to activation of effector cells in both branches of the immune system. T cells and B cells communicate with each other through various interactions involving receptors, e.g., the interaction between the T cell receptor and antigen, or the interaction between various cell adhesion molecules and their ligands, and through the secretion of various soluble factors.

Cell surface adhesion molecules play a role in the function and regulation of the immune response by enhancing the efficiency of interactions between lymphocytes and accessory cells or target cells, promoting interactions between leukocytes and endothelial cells, and by facilitating the recirculation of lymphocytes. Monoclonal antibodies to these adhesion molecules can inhibit the interaction between cells. Various adhesion molecules have been identified, including a lymphocyte function-associated antigen-1 (LFA-1) and the T cell surface markers CD2, CD4, and CD8.

Each adhesion molecule is thought to interact with a specific ligand on the surface of another cell.

Before B cell activation and subsequent antibody production can occur in an immune response, antibody present on the B cell surface must first recognize an antigen, either in soluble form or on the surface of a macrophage. However, in most cases, antigen alone is not sufficient to trigger B cell activation. The cooperation of an activated helper-inducer T cell and lymphokine interleukin-1 (IL-1) is required. The activated helper-inducer T cells can produce soluble helper factors such as IL-4, IL-5, and IL-6, which induce the B cells to proliferate and differentiate into antibody producing cells (plasma cells) or memory cells.

In a normal immune response, activation and proliferation of B cells occurs when antigen interacts with the B cell antigen receptor, or membrane bound immunoglobulin, followed by aggregation, or patching, of these receptors on the cell surface. In addition, other factors are required, such as the presence of T cells and macrophages as well as lymphokines that promote B cell growth and differentiation.

The absence of any of these factors may lead to an undesirable "tolerance". Tolerance in the normal immune system means that it does not respond destructively to self-antigens. An extreme situation occurs when this tolerance expands to non-self, and potentially all, antigens. Another mechanism that can cause such tolerance is ligand-induced inactivation, or antigen blockade. Immune tolerance may also be induced by other mechanisms, including the inhibition of B cell activation either by CD^8+ suppressor T cells or by inhibition of CD^4+ helper-inducer T cell activation.

The other extreme of immune disorders are autoimmune diseases, which are defined as any disease

caused by immunologic reaction to self-antigens, i.e., the normal tolerance is lost or diminished. Organ-specific autoimmune diseases include myasthenia gravis, thyroiditis, primary biliary cirrhosis, arteriosclerosis, and autoimmune hemolytic anemia. Systemic autoimmune diseases include rheumatoid arthritis, systemic lupus erythematosus and rheumatic fever.

Three mechanisms are principally responsible for inflammation and tissue injury in autoimmune disease: cell lysis and release of inflammatory mediators triggered by autoantibodies, immune complex disease, and cell-mediated immunity. In the first mechanism, circulating autoantibodies react with modified or unmodified antigens on cell surfaces. The bound antibodies then stimulate the release of mediators of inflammation, trigger the complement pathway, or activate cytotoxic cells of the immune system. In the second mechanism, complexes between autoantibodies and antigens form in a circulation or in intercellular fluids. These complexes then deposit in various tissues and cause inflammation and tissue injury. In the third mechanism, sensitized T cells either injure cells directly or release lymphokines that amplify the inflammatory response.

Summary of the Invention

We have discovered that CD22β mediates B cell interaction with CD4+, CD8+, and CD16+ (NK cells) T lymphocytes, as well as tonsilar B cells and B cell lines. T and B cell adhesion to CD22β occurs via at least two known different sialylated ligands. The T cell ligand recognized by CD22β is believed to be CD45RO, a cell surface phosphotyrosine phosphatase, associated with the helper T cell phenotype (Smith et al., Immunol., 58:63-70 (1986); Streuli et al., J. Exp. Med., 188:1548-66 (1987); Tonks et al., Biochemistry, 27:8695-701

(1988)); and the B cell ligand is CD75, a cell surface α2-6 sialyltransferase which is highly expressed on activated B cells.

CD22β constitutes the first CD45 ligand to be identified; its interaction with CB22β may regulate T cell activation.

The invention features a method of inhibiting, in a biological sample or system the binding of a CD22β-bearing B cell to a second cell bearing a CD22β-specific ligand, by contacting the sample with a substance which binds to the CD22β-speciifc ligand to competitively inhibit the binding of the B cell to the second cell. This second cell may be a T cell or a B cell.

The preferred form of the inhibiting substance is a soluble protein including a portion of CD22β capable of binding to a CD22β-specific ligand binding site on a T cell. The inhibitory substance may also be an antibody to naturally occurring B cell CD22β.

The invention also features a soluble protein fragment capable of binding to a CD22β-specific ligand on a T cell. Preferably, this fragment excludes the transmembrane region of CD22β or includes only a portion of the transmembrane region small enough not to prevent solubilization of the fragment. In further preferred embodiments, this fragment is at least 70% homologous with a region of CD22β and contains at least 322 amino acids.

The biological system noted above may be a human patient, in which case the method results in inhibition of the immune reponse of that patient.

The invention also features a therapeutic composition including one or more different soluble fragments as defined above in a pharmaceutically acceptable carrier. The invention further features a method of inhibiting a human patient's immune response to

alleviate an autoimmune disease, by administering to the patient an effective amount of this therapeutic composition.

This invention also features an expression vector including a DNA sequence encoding the soluble fragment and a cell comprising that expression vector. A soluble CD22β fragment according to the invention may be made by culturing this cell and isolating the soluble fragment therefrom.

In addition, the invention features methods of competitively inhibiting the binding of B cells to T cells, on other B cells, in a human patient, thereby preventing activation of both T and B cells, by administering an inhibiting amount of a composition including a soluble protein comprising a portion of CD22β capable of binding to a CD22β-specific ligand on a T cell, and a pharmaceutically acceptable carrier.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description

The drawings are first briefly described.

Drawings

Fig. 1a is an autoradiograph showing immunoprecipitation of CD22β v. CD22α.

Fig. 1b is a schematic representing the structures of CD22α and CD22β.

Figs. 2a to 2h are a series of photomicrographs showing CD22β-mediated adhesion of peripheral blood and tonsillar lymphocytes.

Fig. 3 is a set of four schematics representing the structures of truncated forms of CD22β.

Fig. 4 is a graph showing the T and B lymphocyte-binding epitopes of CD22β.

Fig. 5 is a graph showing the blocking of Molt-4 and Daudi cell adhesion to CD22β transfectants by monoclonal antibodies and neuraminidase treatment.

Fig. 6a is a schematic of a soluble CD22β protein.

Fig. 6b is an autoradiograph of two purified CD22β fusion proteins.

Fig. 7a is a graph showing the reactivity of UCLH-1 monoclonal antibody with resting and activated T cells.

Fig. 7b is a graph showing the reactivity of resting T cells with CD22Rg, a CD22-immunoglobulin chimera.

Fig. 7c is a graph showing the reactivity of Molt-4 cells with various CD22-immunoglobulin chimeras.

Fig. 8 is a series of graphs showing the comparison of CD45RO+/CD45RO-peripheral blood T cells and other T cells for reactivity with UCHL-1 and CD22Rg.

Figs. 9a to 9d are a series of graphs showing the reactivity of anti-CD75 monoclonal antibody and CD22Rg with CD75-transfected COS cells and B cell lines and antibody blocking of Daudi cell binding to CD22-β-expressing COS cells.

Fig. 10 is a schematic of the cDNA nucleotide and amino acid sequnces of the entire coding and 3′ untranslated region of CD22β.

Isolation and Characterization of cDNA Clones Encoding CD22β

Molecular cloning of CD22β

Isolation of CD22α has been reported previously. CD22β was isolated from Nalm-6 and Raji cDNA libraries, constructed as described by Stamenkovic and Seed, J. Exp. Med., 167:1975-80 (1988), by polymerase chain reaction (PCR) using synthetic oligonucleotide primers complementary to sequences 5′ and 3′ of the coding region of CD22α designed to include an XhoI site at the 5′ end and a PstI site at the 3′ end:

CD22F: CGC GGG CTC GAG ACG CGG AAA CAG GCT TGC ACC CAG ACA CGA
CD22R: CGC GGG CTG CAG GTC TGG GGA AAA CTC GGG GAC TTC CCT GGC

Reactions were done using amplitaq polymerase (Perkin Elmer) and buffers recommended by the vendor. Thirty cycles of amplification were carried out using the following scheme: 94 oC/1min, 60 oC/2min, 72 oC/3min. Following the amplification, a fraction of the product was examined on a 1% agarose gel, and the remaining product was subjected to restriction nuclease digestion, after phenol-extraction and ethanol-precipitation.

Two PCR products of 2.6 kb and 2.1 kb were obtained from the Raji cDNA library, while PCR products derived from Nalm-6 and Daudi cDNA libraries consisted of a single 2.6 kb and 2.1 kb fragment respectively. Both 2.6 and 2.1 kb amplification products were subcloned into a CDM8 expression vector (Seed, Nature, 329:840-42 (1987)) and introduced into COS cells by the DEAE-Dextran method. Forty-eight hours following transfection, COS cells were tested for monoclonal antibody (mAb) reactivity. Cells transfected with the 2.6 kb PCR amplification product derived from Nalm-6 and Raji cDNA libraries reacted with mAb to all of the reported CD22 epitopes (Schwartz-Albiez et al., Leukocyte Typing IV, Oxford University Press, 65-67 (1989)), while cells expressing the 2.1 kb fragment reacted with only two of the mAb, HD39 and BL3C4, similar to the first reported CD22 cDNA isolate.

Immunoprecipitations

Immunoprecipitations were performed as previously described in Stamenkovic and Seed, Nature, 344:74-77 (1990). Briefly, COS cells were labelled with ^{125}I, washed in PBS buffer, and lysed with a buffer containing a 1% Nonidet-P40, 20 mM iodoacetamide, and 1 mM phenylmethylsulfonyl- fluoride in a Tris-buffered saline solution. The lysates were centrifuged, precleared with

10 μg of isotype matched mouse IgG and protein A beads (Pierce) at 4°C overnight and incubated with 10μg of Leu-14 monoclonal antibody (mAb) and fresh protein A beads for 4 hours at 4°C. Beads were washed in lysis buffer, resuspended in loading buffer containing mercaptoethanol, and eluted by boiling. Eluates were electrophoresed on an 8% polyacrylamide gel and the dried gel was autoradiographed for 24h.

As shown in Fig. 1a, immunoprecipitation of the two isoforms from transfected COS cells revealed that the product of the larger cDNA yielded a 130 kD species, while that of the smaller insert yielded a 110kD band, similar to the previously described CD22 polypeptide.

DNA sequence analysis, by the dideoxy method, of the larger CD22 insert showed an open reading frame identical to the reported CD22β sequence (Wilson et al., supra), predicting an extracellular region composed of 7 Ig-like domains. The sequence of the smaller insert was identical to that of CD22β with the exception that extracellular Ig domains 3 and 4 were deleted (Fig. 1b). The smaller isolate therefore corresponds to CD22α.

CD22β Mediates the Adhesion of CD4+ T Cells and Tonsillar B Cells

To determine whether the two additional Ig-like domains of CD22β provide new adhesion properties compared to CD22α, COS cells transfected with CD22β were incubated with freshly isolated peripheral blood or tonsillar mononuclear cells under conditions previously described in Stamenkovic and Seed, Nature, 344:74-77 (1990).

COS cells were transfected with CD22α, CD22β or CD20 by the DEAE-Dextran method, trypsinized 12 hours after transfection and replated in 6 cm plates at 25% confluence to facilitate rosette scoring, and cultured for 1-2 additional days before performing the adhesion assays. Peripheral blood and tonsil mononuclear cells

were separated on Ficoll Hypaque gradients, washed several times in PBS, and resuspended in Dulbecco's modified Eagle's medium (DMEM) in the presence of heparin (500 U/ml). PHA blasts were obtained by incubating Ficoll-Hypaque separated peripheral blood mononuclear cells with 1 μg/ml PHA for 72 hours at 37°C in RPMI supplemented with 10% fetal bovine serum. 48 hours following transfection, COS cells were overlayed with PBL, tonsillar cells, or PHA blasts in 2 ml DMEM and 500 U/ml heparin, and incubated at 4°C for 30 min. Non-adhering cells were removed by gentle washing with PBS and the remaining cells stained with fluorescein- or phycoerythrin labelled anti-CD3 (leu4), anti-CD4 (leu-3a), anti-CD8 (leu2), anti-CD14 (leu-M3), and anti-CD20 (leu-16) (Becton-Dickinson) mAb for 30 minutes at 22°C, washed in PBS, fixed in 4% formaldehyde and examined by fluorescence microscopy.

As shown in Fig. 2, mononuclear cell rosettes were observed around CD22β transfected COS cells, but not around COS cells transfected with unrelated cDNA clones (data not shown). Treatment with fluorescein- or phycoerythrin-labeled monoclonal antibodies to the T cell, myeloid and B cell-specific antigens CD3, CD14 and CD20, respectively, revealed that PBL rosettes were composed predominantly of CD3+ cells, and some CD14+ cells (Figs. 2a and b) but virtually no CD20+ cells (Figs. 2c and d). Unlike PBL rosettes, tonsillar lymphocyte rosettes, which were also largely composed of CD3+ cells, contained a significant number of CD20+ cells (Figs. 2e and f). All of the rosetting T cells, whether derived from PBLs or tonsils, belong to the CD4+ subset (Figs. 2g and h).

These observations indicate that one or both of the two additional domains of CD22β are required for B and T lymphocyte adhesion, but that the presence of these

domains does not inhibit monocyte binding (Figs. 2a and b) or erythrocyte attachment (data not shown), which are presumably mediated by domains common to the two CD22 polypeptides.

Several T cell lines, including Hut 78, Jurkat, and HPB-ALL, failed to adhere to CD22β expressing COS cells. One exception, however, was the T cell leukemia Molt-4, which displayed specific binding. Similarly, a cell line of B cell lineage, the Burkitt lymphoma Daudi, specifically adhered to CD22β transfectants. Neither Molt-4 nor Daudi cells adhered to CD22α-expressing COS cells (data not shown), which is consistent with the observations that CD22-mediated T and B lymphocyte adhesion requires the presence of the two additional CD22β domains.

Construction and Expression of Truncated Forms of CD22

To identify the domains of CD22β responsible for antibody and PBL binding, truncated forms of CD22β comprising sequences encoding single or multiple Ig-domains of CD22β were ligated to sequences encoding the transmembrane and cytoplasmic sequences of CD32 as described in Stengelin et al., EMBO, 7:1053-59 (1988), and inserted into CDM8 expression vectors. Fig. 3 shows the structure of four such truncated forms of CD22β, along with the predicted amino acid sequences at the sites of fusion.

CD22 cDNA sequences were amplified by PCR using synthetic oligonucleotides complementary to sequences flanking the cDNA regions to be amplified. Oligonucleotides were designed to allow the creation of restriction endonuclease cleavage sites at the 5′ and 3′ extremities of each amplified cDNA segment to facilitate subsequent insertion into CD32 expression vectors. 30 cycles were conducted consisting of 1 min. at 94°C, 2 min. at 60°C, and 3 min. at 72°C, using the reaction buffer recommended by the vendor (US Biochemical). A CD22 primer encoding sequences at the 5′ extremity of the signal peptide and including an Xho I site was synthesized as follows:

5′-CGC GGG CTC GAG ATG CAT CTC CTC GGC CCC TGG CTC-3′

Reverse primers containing a Bgl II restriction site were synthesized with the following sequences:

CD22D1:5′-CTC GAG ATC TTC AGA GAC ATT GAG GTG TAT TCG TTC-3′
CD22D2:5′-CTC GAG ATC TTT CAC GTT CAG CTG CAC CGT GTC ATT-3′
CD22D3:5′-CTC GAG ATC TTC CGG GGC ATA CTG CAC TTG CAG GAA-3′
CD22D4:5′-CTC GAG ATC TGT GGT CAC CTT CTT GGG AGG ATA CTG GAC-3′

CD22 PCR products were digested with Xho I and Bgl II and ligated to Xho I-BamHI-cut CD4-CD32 vector.

Constructs containing CD22 Ig-domains 1; 1 and 2; 1, 2, and 3; and 1, 2, 3, and 4, were expressed in COS cells and tested for mAb binding. The reactivity results are shown in Fig. 3, on the right side. Domain 1 failed to show reactivity with anti-CD22 mAb, whereas domains 1 and 2 reacted with mAb Leu-14 and B1-3C4, which are thought to recognize two different CD22 epitopes (Schwarz-Albeiz et al., supra), and supported erythrocyte adhesion (data not shown). Constructs containing CD22 domains 1-3 and 1-4 were expressed in COS cells, reacted with all anti-CD22 mAb, and mediated adhesion of both Molt-4 and Daudi cells. Sequences required for reactivity with known antibodies, as well as T and B cell adhesion, are therefore encoded in the first three CD22 Ig-like domains (residues 1-302).

These results demonstrate that B and T cell binding sequences are encoded in the first three Ig domains of CD22β.

T and B Cell Lines Bind to Different Epitopes of CD22β

To determine whether T and B lymphocyte adhesion is mediated by the same or different CD22 epitopes, blocking assays were performed by treating CD22β-transfected COS cells with a panel of anti-CD22 mAb, prior to incubation with Molt-4 or Daudi cells. Two of the mAbs, Leu-14 and B1-3C4 mAb, which recognize both CD22 polypeptides, failed to inhibit adhesion of either cell line. However, Molt-4 adhesion was completely blocked by pre-incubation of COS cell transfectants with CD22-specific mAb IS7 and To15, which recognize two distinct CD22 epitopes (Schwarz-Albiez, et al., supra), whereas Daudi cell adhesion was blocked by To15 only (Fig. 4). Two additional mAbs, HD6 and OTH228, specific for CD22β, had no inhibitory effect on either Molt 4 or Daudi cell adhesion. These observations suggest either that Molt-4 and Daudi cell attachment is mediated by

different epitopes of the CD22β molecule or that two CD22β epitopes are required for T cell binding while a single epitope, which constitutes part of the T cell binding site, is sufficient for B cell adhesion. In either case, it appears likely that T and B cells may express different ligands for CD22β.

CD22β-Mediated T Cell Adhesion
is Blocked by the CD45RO mAb UCHL-1

T cells adhering to CD22β-transfected COS cells are CD4+, but not all CD4+ lymphocytes bind to CD22β-transfectants (data not shown). To identify the T cell ligand of CD22, a panel of mAb to cell surface antigens expressed on subpopulations of CD4+ T cells were tested for blocking adhesion of Molt 4 cells to CD22β-transfected COS cells. The panel included mAb to CD2, CD4, CD5, CD6, CD7, CD8, CD18, CD44 and CD45 antigens. Only one monoclonal antibody, UCHL-1, which recognizes the restricted leukocyte common antigen isoform CD45RO (Smith et al., Immunol., 58:63-70 (1986); Terry et al., Immunol., 64:331-36 (1988)), specifically blocked adhesion of Molt-4 cells (Fig. 5). Similarly, pre-incubation of tonsillar cells, PBLs and day 3 PHA-blasts with UCHL-1 resulted in blocking of T cell adhesion to CD22 transfectants (data not shown). Monoclonal antibodies 2H4 and 4KB5, specific for the CD45A isoform (Streuli et al., J. Immunol., 141:3910-17 (1988); Schwinzer, Leukocyte Typing IV, Oxford University Press, 628-37 (1989)), did not inhibit Molt-4 (Fig. 5), peripheral blood, or tonsillar T cell binding. The different lanes in Fig. 5 show the percentage of adhesion (compared to medium only) of Daudi and Molt-4 cells to CD22β transfectants in the presence of: lane 1, no treatment; lane 2, anti-CD44 mAb; lane 3, anti-CD5 mAb; lane 4, anti-CD8 mAb; lane 5, anti-CD45 mAb 2H4; lane 6,

anti-CD45 mAb 4KB5; lane 7, anti-CD45 mAb UCHL-1; and lane 8, neuraminidase.

Monoclonal antibody assays

Transfected COS cells were pre-incubated with 50 μg of each anti-CD22 mAb for 45 min. at 22°C, washed in PBS, and overlayed with $5x10^6$ Molt-4 or Daudi cells in DMEM with 500U/ml heparin. Incubation proceeded for 30 min. as above. Rosettes were scored by recording the number of mononuclear cells in each individual rosette. Because of variation in COS cell size, 100-200 COS cells were scored per assay. Average scores obtained for adhesion in the presence of each mAb are expressed as a percentage of average scores obtained for adhesion in the presence of media alone.

Monoclonal antibodies to CD2, CD4, CD4, CD6, CD7, CD8, CD18, CD44, CD45 and CD75 were obtained from the Fourth International Typing Leukocyte Workshop. Antibodies to CD45RO (UCHL-1) and CD45RA (2H4) were a kind gift of Dr. Stuart Schlossman. Antibody blocking was performed by pre-incubating transfected COS cells or PBL, TL, PHA blasts and cell lines with monoclonal antibodies at a concentration of 50μg/ml for 45 min. at 22°C. Cells were washed in PBS and adhesion assays conducted as described above.

Neuraminidase blocking assays

Recent reports have shown that presence of sialic acid groups on CD45RO is required for reactivity with UCHL-1 mAb (Pulido and Sanchez-Madrid, Eur. J. Immunol., 20:2667-71 (1990)). Neuraminidase treatment of Molt 4 cells abolished UCHL-1 reactivity (data not shown), and abrogated adhesion to CD22-β transfected COS cells (Fig. 5), suggesting that sialylation of CD45RO is critical for interaction with CD22β as well. Reactivity of neuraminidase treated cells with monoclonal antibodies specific for other cell surface glycoproteins, including

CD7 and CD44 was unaltered (data not shown), suggesting that the observed loss of adhesion was not due to neuraminidase-induced cell damage.

Neuraminidase treatment was done by incubating $5x10^6$ cells with 50 mU/ml neuraminidase from Vibrio cholerae at 37°C for 30 min. To quantitate the adhering cells, transfected COS cells were treated with a non-blocking anti-CD22 mAb (leu-14) at a dilution of 1:500 in PBS, for 30 min. at 22°C immediately following the adhesion assay. The cells were rinsed with PBS, incubated with a fluorescein-conjugated goat anti-mouse affinity purified antibody (Cappel), rinsed with PBS, fixed in 4% formaldehyde and examined under a fluorescence microscope. The number of mononuclear cells forming rosette around each leu-14-positive COS cell was recorded. In each individual adhesion assay, 200 COS cells positive for Leu-14 reactivity were scored. No rosettes were observed with leu-14 negative COS cells.

Soluble CD22-Ig Fusion Proteins React With CD45RO

To provide more direct evidence that CD22β interacts with CD45RO, soluble CD22 Ig chimeras, which we refer to as CD22Rg (Aruffo et al., Cell, 61:1303-13 (1990)), were created by genetic fusion of cDNA segments encoding the first three (CD22D3) or four (CD22D4) extracellular Ig-like domains of CD22β to genomic DNA segments encoding human IgG1. Fig. 6a shows the structure of these two soluble fusion proteins, CD22D3Rg and CD22D4Rg. Both CD22Rg fusion proteins were efficiently secreted by COS cells and reacted with anti-CD22 monoclonal antibodies HD39 and HD6 (data not shown). Fig. 6b shows the molecular weight of these two proteins on an autoradiograph.

A CD8 fusion protein, described in a previous study (Aruffo et al., supra), was used as a control for non-specific, Fc-mediated interactions. All fusion

proteins formed disulfide-linked dimers similar to immunoglobulins, and accumulated to concentration of 0.5-2 μg/ml in COS cell supernatants at 7-10 days post transfection. To test for ligand reactivity, fusion proteins were used as supernatants or after purification on a protein A-sepharose column.

Purified CD22Rg (continuous line) but not CD8Rg (dotted line) revealed reactivity with Molt-4 cells (Fig. 7c). The reactivity was specific, since no binding was observed to several CD45RO-negative T cell lines, including the T cell leukemias Hut 78 and HPB-ALL (Fig. 8), and was blocked by incubation with UCHL-1 (discontinuous line)but not with 2H4 mAb (dash-dotted line)(Fig. 7c). Because expression of CD45RO in T cells is enhanced upon activation, UCHL-1 and CD22Rg reactivity of resting T cells (a), day 3 PHA-blasts (b) and T cells cultured for 16 days following PHA stimulation (c) were compared (Figs. 7a and b). Not only was the percentage of UCHL-1-reactive, resting T cells, day 3 PHA blasts and 16-day post-PHA stimulation T cells (71%, 84% and 93%, respectively) comparable to CD22Rg reactive fractions (69%, 81% and 88% respectively), but the relative intensity of reaction with CD45Rg of all three T cell populations (Figs. 7a and b), was a reflection of the level of CD45RO expression. The similar intensity of staining with UCHL-1 and CD22Rg, however, does not reflect similar affinity for CD45RO, since CD22Rg was used at concentrations of 45-50 μg/ml compared to 5 μg/ml of UCHL-1 mAb or a 1:200 dilution of ascitic fluid.

To provide further support for the suggestion that CD45RO interacts with CD22β, purified peripheral blood T cells were sorted into CD45RO+ and CD45RO- subpopulations and tested for CD22Rg reactivity and CD22β-mediated adhesion. Only CD45RO+ cells were observed to react with

CD22Rg and to form rosettes with CD22β-expressing COS cells (Fig. 8).

Production of soluble CD22

cDNA sequences encoding the first 3 or 4 Ig-like domains of CD22 were amplified by PCR and the amplified sequences were ligated to plasmids containing genomic sequences encoding the Fc portion of IgG1 as previously described in Aruffo et al., Cell, 61:1303-13 (1990). CD22.3Rg and CD22.4Rg constructs were introduced into COS cells by the DEAE-Dextran method, and supernatants collected 5-7 days post transfection. Supernatants were tested for CD22Rg production by labeling COS cells with 35S-cysteine-methionine (ICN) and precipitating the labeled soluble CD22 with protein A beads (Pierce). For purification, supernatants were passed over a protein A trisacryl column (Pierce) (typically 250 µl of packed protein A beads were used for 200 ml of supernatant) at room temperature. Protein was eluted in 0.1 M acetic acid, pH 4.5 and immediately neutralized in tris buffer to a pH of 8.0. For staining reactions, soluble CD22Rg was used at concentrations of 25-50 µg/ml.

Monoclonal antibody and soluble CD22 reactivity with T cells and T and B cell lines

Purified peripheral T cells were obtained by depleting Ficoll-Paque-separated mononuclear cells of B cells using anti-human IgM antibody-coated magnetic beads (Dynal, Oslo, Norway) and of monocytes by panning on plastic dishes for 30 min. A 95% pure CD3+ population was thus obtained. Purified T cells and T and B cell lines were incubated with monoclonal antibodies at 5 µg/ml, or 1:200 dilution of ascitic fluid, or CD22Rg and CD8Rg at 25-50 µg/ml, as above. Antigen expression and CD22Rg reactivity were measured by indirect fluorescence and flow cytometry. Purified peripheral T cells were sorted into CD45RO+ and CD45RO- populations on an EPICS cell sorter.

CD22Rg Blocks Anti-CD3-Mediated T Cell Activation

Because CD45 is thought to be involved in regulating protein phosphorylation, interaction of CD45RO with CD22 may play a role in T cell activation. Preliminary studies designed to determine a possible functional role of CD22 in T cell triggering, revealed that anti-CD3 mediated T cell activation is blocked by CD22Rg in a dose-dependent fashion (Table 1). Anti-CD45RO mAb UCHL-1 produced a less pronounced effect in soluble form at comparable doses but had a strong inhibitory effect when crosslinked to plates. Human immunoglobulins and CD8Rg at comparable doses produced no effect on T cell activation (Table 1). Unlike UCHL-1, CD22Rg did not require crosslinking to block CD3-mediated activation. Neither soluble CD22Rg nor soluble or plated UCHL-1 mAb had any effect on PHA-mediated T cell activation (data not shown), consistent with the notion that triggering of CD45RO by antibody or ligand modulates some but not all T cell activation pathways. The amounts of antibody and CD22Rg used are indicated on Table 1. All assays were done in triplicate or quadruplicate. The anti-CD3 MAb used for T cell stimulation was 12F6 as described by Wong et al., J. Immunol., 143:3404-11 (1989).

Although further studies are required to determine the physiologic effects of CD22β on T cells, our data indicates that interaction between CD22β and its T cell ligand down-regulates T cell activation.

Table 1. Inhibition of T Cell Activation by CD22 Rg

Stimulation with Anti-CD3 (Plated) (10 μg/ml)	Antibody or Soluble Receptor--Immunoglobulin Added (40 μg/ml)				Mean Proliferation ± SD (cpm)
	Human IgG	UCHL-1	CD8Rg	CD22Rg	
-	-	-	-	-	1,159 ± 174
+	-	-	-	-	95,441 ± 15,938
+	-	+	-	-	67,723 ± 5,221
+	-	-	+	-	102,755 ± 7,375
+	-	-	-	+(40μg)	13,960 ± 3,731
+	-	-	-	+(80μg)	1,636 ± 240
+	+	-	-	-	91,375 ± 5,961
+	-	+(Plated)	-	-	7,509 ± 3,631

The B Cell Ligand for CD22
is the α2-6 Sialyltransferase CD75

Similar to Molt 4 cells, Daudi cell adhesion to CD22-transfected COS cells was abrogated by neuraminidase treatment. However, B cells do not express CD45RO (Thomas, Ann. Rev. Immunol., 7:339-69 (1989)), and Daudi cells displayed no reactivity with UCHL-1 mAb (Fig. 9c). These observations suggest that a sialylated glycoprotein distinct from CD45RO is likely to be the B cell ligand for CD22-β. B cells express several sialylated cell surface proteins, prominent among which are the low affinity IgE receptor, CD23 (Barsoumian et al., Leukocyte Typing IV, Oxford University Press, 110-12 (1989)), sialophorin/CD43 (Stross et al., id., 615-17), different isoforms of CD45 (Thomas, Ann. Rev. Immunol., 7:339-69 (1989)), and α2-6 sialyltransferase/CD75 (Stamenkovic et al., J. Exp. Med., 172:641-43 (1990)). CD22Rg (continuous line) specifically reacted with COS (Fig. 9b) and Hela (data not shown) cells transfected with a CD75-specific cDNA, but not with COS cells transfected with cDNA clones encoding CD23, (Stamenkovic and Seed, unpublished) and CD43 (Stamenkovic, unpublished) (data not shown).

Fig. 9a shows the reactivity of CD75-transfected COS cells with anti-CD75 mAb HH-2 (continuous line) and with an unrelated, isotype-matched mouse antibody (dotted line). Fig. 9b shows the reactivity of these same transfected COS cells with CD22Rg (continuous line), CD22Rg following treatment with neuraminidase (dotted line), and CD8Rg (sparcely dotted line).

The observation that human CD75, introduced into both simian fibroblasts and human epithelial cells, preserves reactivity with CD22Rg is consistent with direct interaction between the two molecules. However, the recent discovery that CD75 is identical to α2-6

sialyltransferase (Stamenkovic et al., 1990, supra), raises the possibility that the observed CD22Rg reactivity may be due to α2-6 sialylation of an intrinsic COS or Hela cell surface molecule as a result of CD75 expression. To eliminate this possible explanation, COS cells transfected with unrelated cDNA clones were incubated with varying concentrations of soluble sialyltransferase, which, in the presence of appropriate substrate, has been shown to retain its enzymatic activity (Weinstein et al., J. Biol. Chem., 257:13835-44 (1982)). If CD22Rg recognizes a resident α2-6 sialylated COS cell receptor, CD22Rg reactivity with COS cells subjected to soluble sialyltransferase would be expected. However, soluble sialyltransferase failed to induce COS cell reactivity with CD22Rg.

To determine whether soluble sialyltransferase effectively sialylates COS cell antigens, COS cells treated with soluble sialyltransferase or transfected with CD75 were treated for agglutination with sambucus nigra bark lectin (SNA). SNA specifically agglutinates α2-6 sialylated glycoproteins (Shibuya, et al., Arch. Biochem. Biophys., 254:1-8 (1987)), but has virtually no effect on untreated COS cells, suggesting that COS cells do not constitutively express significant levels of α2-6 sialylated molecules. CD75-transfected and soluble sialyltransferase-treated COS cells both displayed agglutination in the presence of SNA (data not shown), indicating that both the cell surface form and the soluble form of sialyltransferase mediate sialylation of COS cell glycoproteins.

To determine how CD75 expressed in COS cells compares to B cell CD75 for reactivity with CD22Rg, mature and lymphoblastoid B cell lines were compared for CD75 expression, CD22Rg reactivity, and CD22β-mediated adhesion. Daudi and Raji cells revealed CD22Rg

reactivity which reflected CD75 surface expression, and, accordingly, formed rosettes with CD22-β-transfected COS cells (Fig. 9c). The B lymphoblastoid cell line IM-9, which lacks CD75 expression, failed to adhere to CD22-β transfectants or to react with CD22Rg (Fig. 9c). Fig. 9c also shows the use of murine antibodies (dotted lines) as controls for UCHL-1 and HH-2 mAbs reactivity, and CD8Rg as a negative control (dotted line) for CD22Rg binding. The mAbs were used at 5 μg/ml and CD22Rg and CD8Rg were used at 50 μg/ml.

Additional support for physical association between CD22-β and CD75 was provided by the observation that the CD75-specific mAb HH-2 (Erikstein et al., Leukocyte Typing IV, Oxford University Press, 110-12 (1989)) blocked Daudi cell adhesion to CD22β-transfected COS cells (Fig. 9d). Fig. 9d shows no blocking by the medium, or the mAbs 2H4, which recognizes CD45RA, UCHL-1, or IF5, which recognizes CD20. Taken together, these results suggest that CD22Rg binds CD75 directly.

Sialyltransferase functional assay

To test for sialyl transferase activity, COS cells treated with soluble sialyltransferase (Sigma), transfected with CD75 or untreated, were lifted off the culture plates with 0.5 mM EDTA, washed in PBS, incubated with 10 μg/ml, and overlayed with treated, transfected, or untreated COS cells. Adherent cells were counted after a brief incubation of 10-15 minutes at room temperature.

To test for soluble sialyltransferase induction of CD22Rg reactivity, COS cells transfected with an unrelated cDNA, encoding CD20, were treated with soluble sialyl transferase (Sigma, St. Louis, MO) at concentrations from 0.01 mM to 1 mM in DMEM/10%FBS for 30 min. to 2 hr. at 37°C, in the presence of CMP-sialic acid (Sigma) according to procedures of Weinstein et al., J.

Bio. Chem., 257:13835-44 (1982). COS cells treated with soluble sialyltransferase were compared for agglutination with CD75-transfected cells and untreated cells or tested for reactivity with CD22Rg by indirect immunofluorescence.

Cell Cultures

In proliferation assays, peripheral blood mononuclear cells isolated on Ficoll-Paque were used. Cells were cultured in quadruplicate samples in 96 well microtiter plates at $2x10^5$/ml, in RPMI medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum and gentamycin. Prior to T cell stimulation, plates were coated with anti-CD3 mAb at a concentration of 10 μg/ml in PBS overnight at 4°C. After 3 days in culture, cells were pulsed for 6 hours with 0.5μCi/[^{3}H] thymidine/well. Cells were harvested with an automatic cell harvester and radioactivity was measured in a liquid scintillation counter. In blocking studies, UCHL-1 mAb, human IgG, CD22Rg and CD8Rg were used at 40 μg/ml or as indicated.

Binding of soluble CD22β to transfected COS cells

COS cells transfected with CD20, CD22β, CD23, CD43, CD44, and CD75 were incubated with purified CD22Rg (25-50 μg/ml) for 1 hour at room temperature, rinsed with DMEM without serum, incubated with fluorescein-labeled affinity-purified goat-anti-human antibody for 30 min. at room temperature, rinsed, fixed in 4% formaldehyde and examined under a fluorescence microscope or by FACS scan.

Production of Soluble CD22β Protein Fragments

Fig. 10 shows the nucleic acid sequence and corresponding amino acid sequence for the CD22β cDNA and protein, as described in Wilson et al., supra.

The CD22β gene may be digested with restriction enzymes to generate a desired DNA fragment; the fragment may then be cloned, expressed, and the resulting protein fragment purified, all according to conventional techniques; e.g., see Maniatis et al., Eds., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor, NY (1982), and Pouwels et al., Eds., Cloning Vectors, Elsevier, Amsterdam (1987). Alternatively, the nucleotide sequence shown in Fig. 10 may be used to generate synthetic DNA molecules encoding either a desired region of the CD22β protein or the complete protein, and the synthetic DNA may then be cloned, expressed, and the protein or protein fragment purified according to conventional techniques. If the entire protein is produced in this way, it may be digested with proteolytic enzymes to generate the desired fragment. Finally, the deduced amino acid sequence of CD22β as shown in Fig. 10, may be used to generate a synthetic peptide.

Use

CD22β interacts with different ligands on T, B, and myeloid cells. The nature of CD45RO, the T cell ligand of CD22β, provides some clues as to the effects of CD22β-mediated adhesion of T cells. CD45RO/CD4 T cells are known to provide help for B cell antibody production (Smith et al., Immunol., 58:63-70 (1986). Furthermore, CD45 molecules display phosphotyrosine phosphatase activity and are thought to regulate signal transduction in lymphocytes by enhancing or blocking cell activation induced through T or B cell surface antigens. The regulatory function is believed to result from interaction between intracellular portions of CD45 and various lymphocyte cell surface molecules. For example, cross-linking of CD45 with CD3 or CD2 inhibits the ability of anti-CD3 and anti-CD2 mAb to increase

intracellular calcium fluxing and stimulate T cell activation. Conversely, cross-linking of CD45 with CD4 greatly augments the calcium fluxing produced upon cross-linking CD4 alone. Ledbetter et al., Proc. Nat. Acad.Sci. USA, 85:8628-32 (1988). CD22β is the first CD45 ligand to be identified and apparently triggers CD45 to regulate T cell activation, and possibly, cytokine production.

Consequently, soluble fragments or analogs of CD22β may be used in the therapeutic regulation of T cell activation. By administering an amount of such a protein effective to competitively inhibit the binding of naturally occurring B cell CD22β to the CD45 sites on a patient's T cells, T cell activation can be down-regulated, thus down-regulating the patient's system. Thus the invention provides a means for treatment of autoimmune diseases, e.g., rheumatoid arthritis, SLE, and Type I diabetes as well as allograft rejection, graft versus host disease, and other disease states in which it is advantageous to inhibit T-cell activation and/or T-cell activation of a B cell humoral response. In addition to soluble CD22β fragments, therapy according to the invention can also employ anti-CD22β antibodies, or antibodies to the T-cell or B-cell ligand for CD22β.

Soluble proteins or protein fragments, as well as antibodies, may be administered to a human patient in one of the conventional modes, e.g., orally, intravenously, parenterally, or transdermally in a sustained release formulation using a biodegradable biocompatible polymer, admixed with an appropriate pharmaceutically acceptable carrier or diluent, or by using micelles, gels, or liposomes.

The soluble protein or antibody can be administered to a human patient in a dosage of about 0.5 mg/kg/day to about 3.0 mg/kg/day.

The use of soluble CD22β may provide additional benefits in treating, e.g., alloqraft rejection, because its use does not cause the body to generate additional antibodies, because the soluble CD22β is recognized as a self-antigen.

Other embodiments are within the following claims.

Claims

1. Use of a substance which binds to a CD22β-specific ligand on a cell in the preparation of a medicament for inhibiting, in a biological sample or system, the binding of a CD22β-bearing B cell to said cell bearing a CD22β-specific ligand.

2. The use of claim 1 wherein said cell bearing a CD22β-specific ligand is a T cell or a B cell.

3. The use of claim 1 wherein said inhibiting substance comprises a soluble protein comprising a portion of CD22β capable of binding to a CD22β-specific ligand binding site on a T cell.

4. The use of claim 2 wherein said inhibitory substance is an antibody to naturally occurring B cell CD22β.

5. A soluble protein fragment capable of binding to a CD22β-specific ligand on a T cell.

6. The fragment of claim 5, excluding the transmembrane region of CD22β or including only a portion of said transmembrane region small enough not to prevent solubilization of said fragment.

7. The fragment of claim 6, said fragment being at least 75% homologous with a region of CD22β.

8. The fragment of claim 7, said fragment containing at least 332 amino acids.

9. The use of claim 1, wherein the biological system is a human patient.

10. A therapeutic composition comprising one or more different soluble fragments as defined in claim 5 in a pharmaceutically acceptable carrier.

11. An expression vector comprising a DNA sequence encoding the soluble fragment of claim 5.

12. A cell comprising the expression vector of claim 11.

13. A method of making a soluble CD22β fragment comprising culturing the cell of claim 12 and isolating said soluble fragment therefrom.

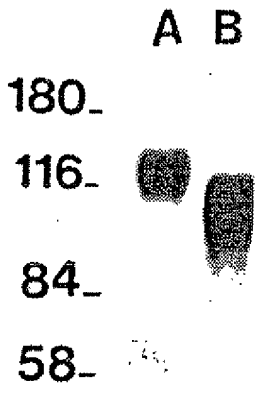

FIG. 1a

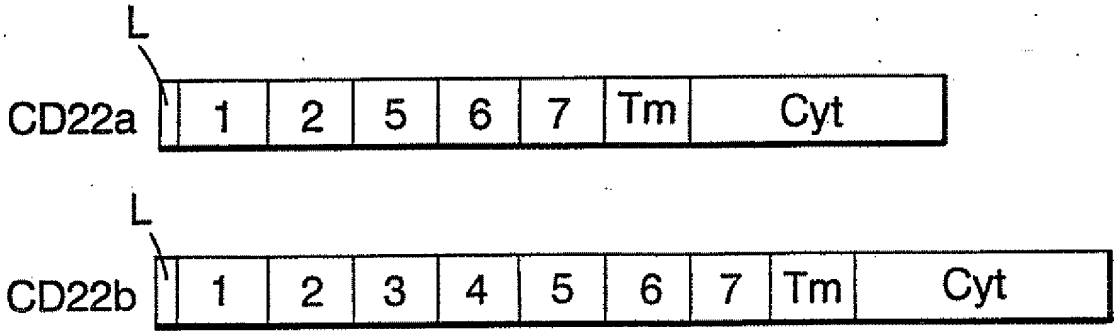
L
CD22a
1
2
5
6
7
Tm
Cyt
L
CD22b
1
2
3
4
5
6
7
Tm
Cyt

FIG. 1b

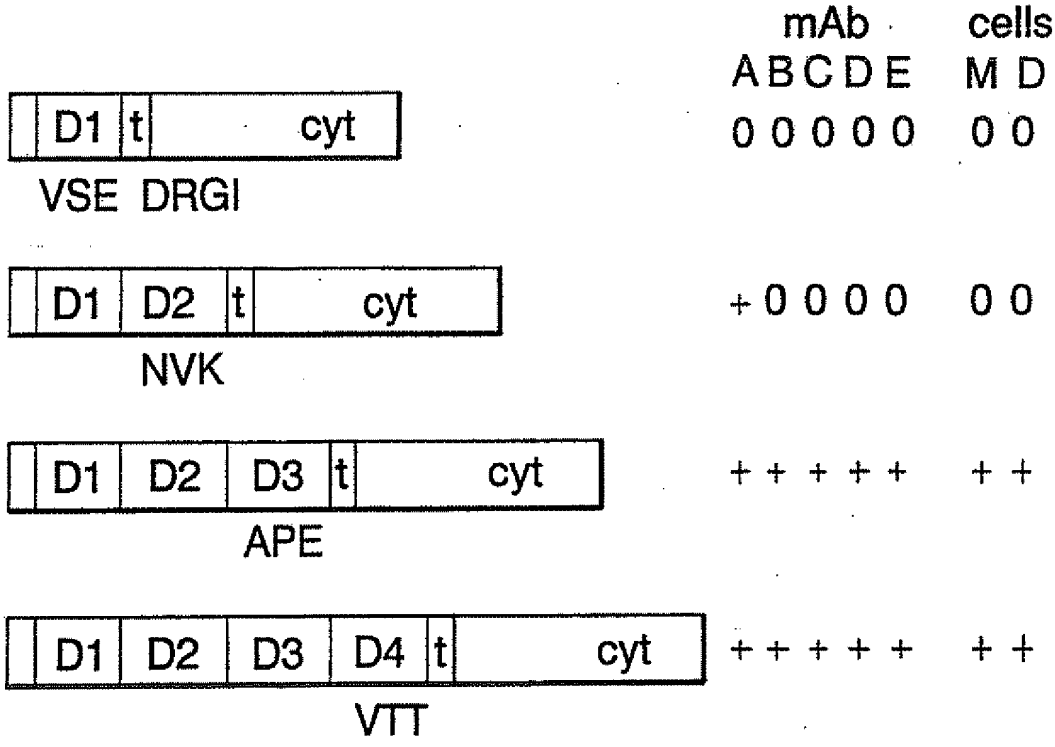
mAb
cells
A B C D E
M D
D1 t cyt
0 0 0 0 0
0 0
VSE DRGI
D1 D2 t cyt
+ 0 0 0 0
0 0
NVK
D1 D2 D3 t cyt
+ + + + +
+ +
APE
D1 D2 D3 D4 t cyt
+ + + + +
+ +
VTT

FIG. 3

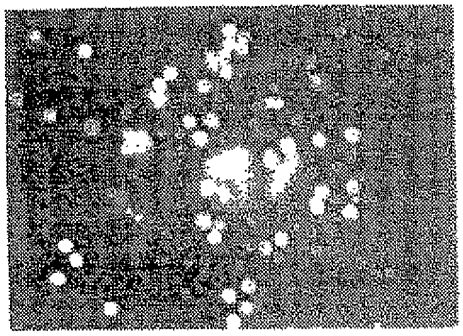

FIG. 2a

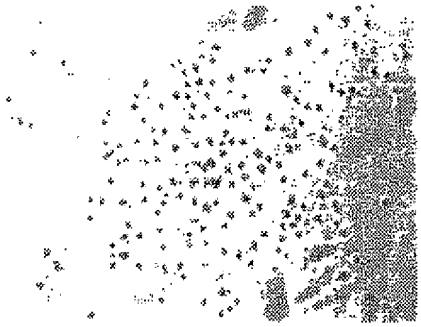

FIG. 2b

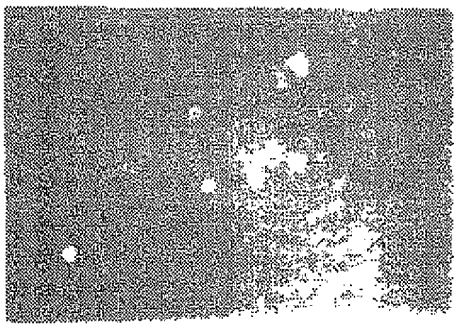

FIG. 2c

FIG. 2d

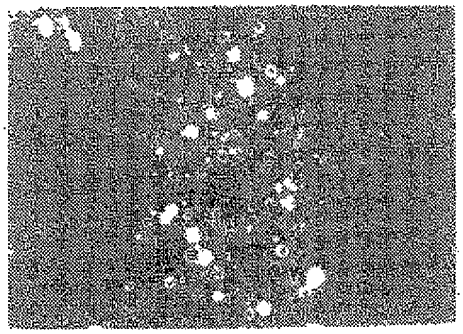

FIG. 2e

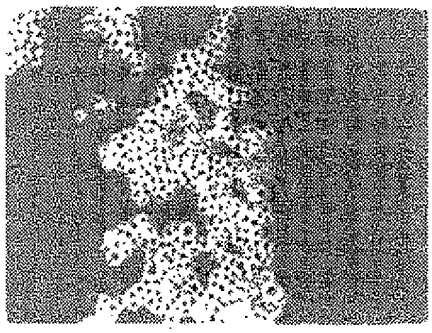

FIG. 2f

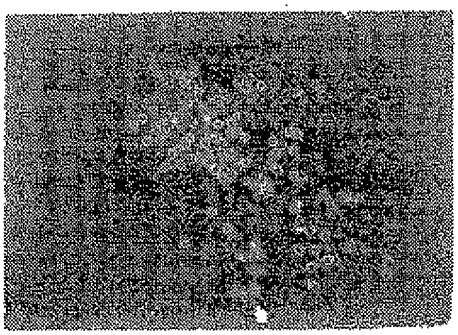

FIG. 2g

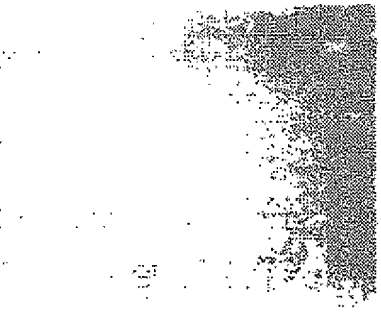

FIG. 2h

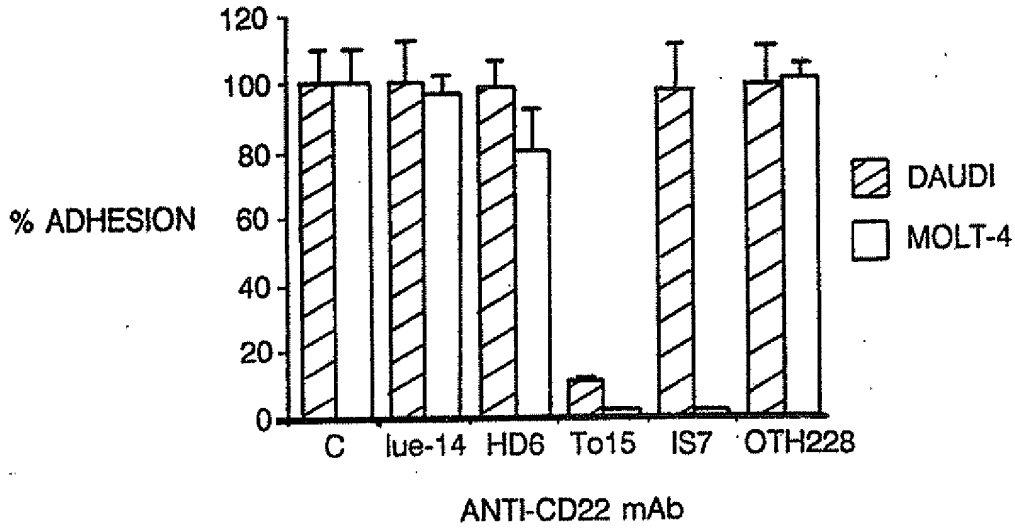
120
100
80
60
40
20
0
% ADHESION
C
lue-14
HD6
To15
IS7
OTH228
ANTI-CD22 mAb
DAUDI
MOLT-4

FIG. 4

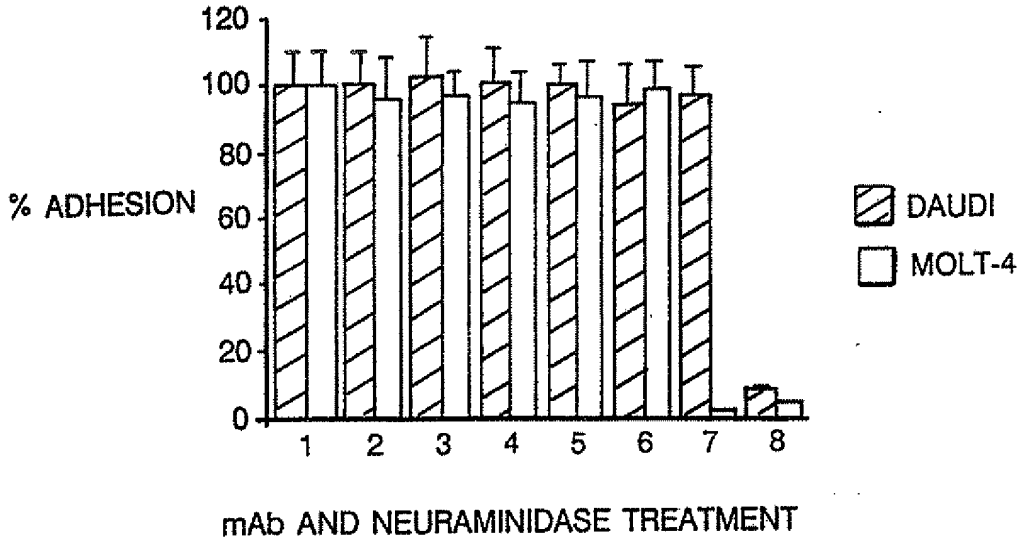
120
100
80
60
40
20
0
% ADHESION
1
2
3
4
5
6
7
8
mAb AND NEURAMINIDASE TREATMENT
DAUDI
MOLT-4

FIG. 5

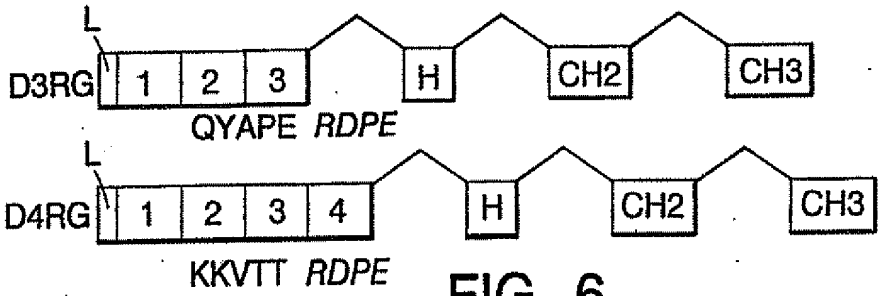

FIG. 6

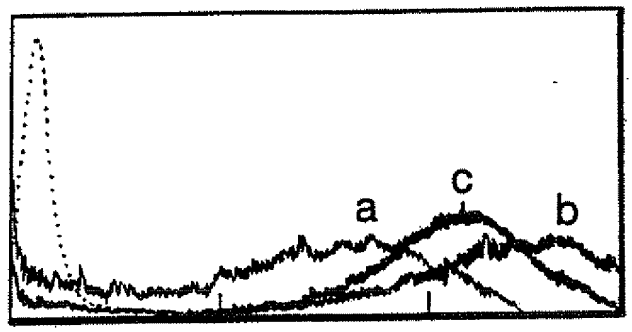

FIG. 7a

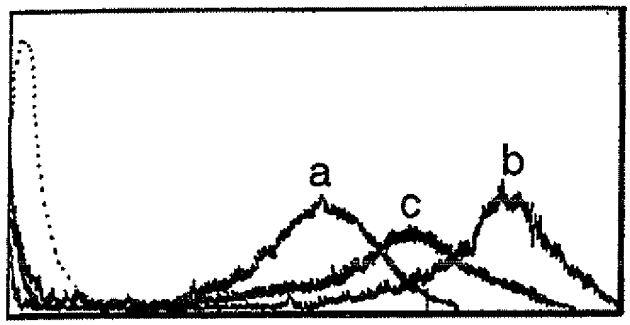

FIG. 7b

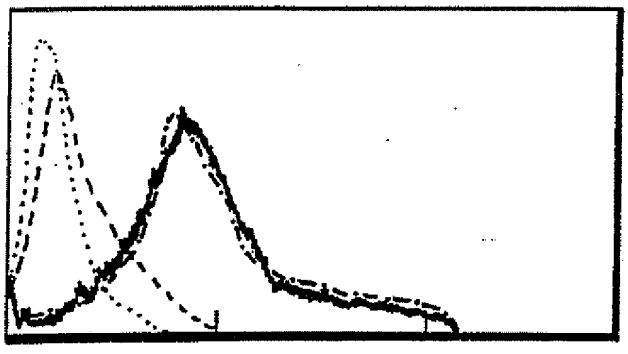

FIG. 7c

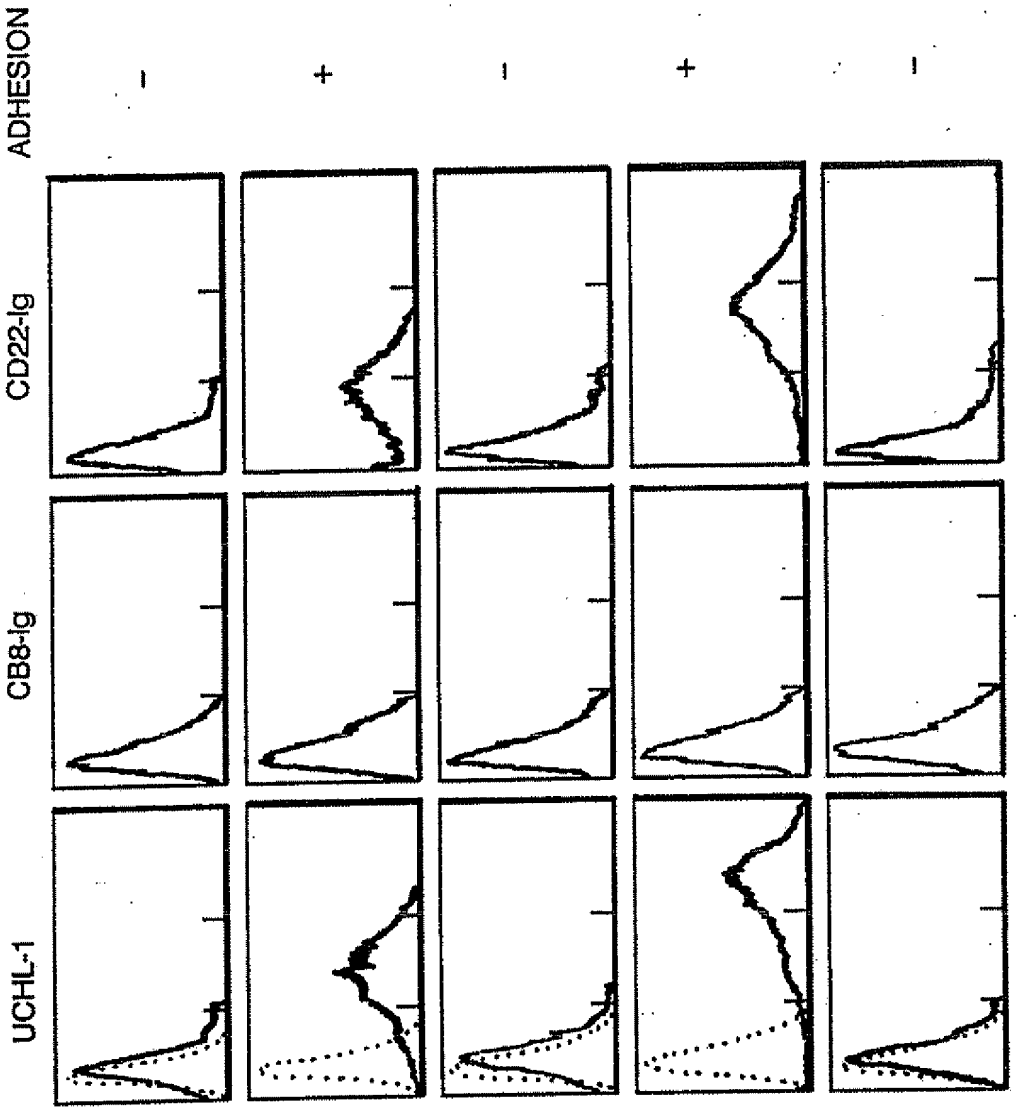
ADHESION
CD22-Ig
CB8-Ig
UCHL-1
-
+
-
+
-
Hut 78
Molt-4
HPB-ALL
T CD45RO+
T CD45RO-
LOG FLUORESCENCE INTENSITY

FIG. 8

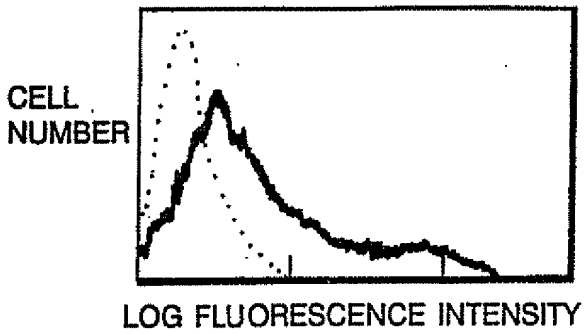

FIG. 9a

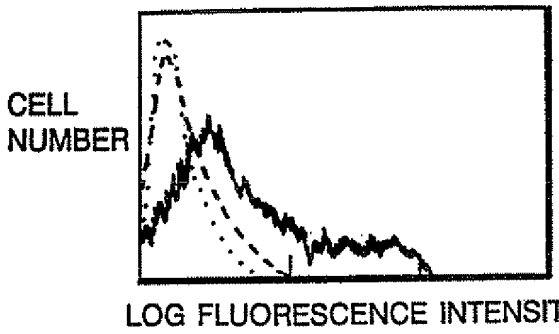

FIG. 9b

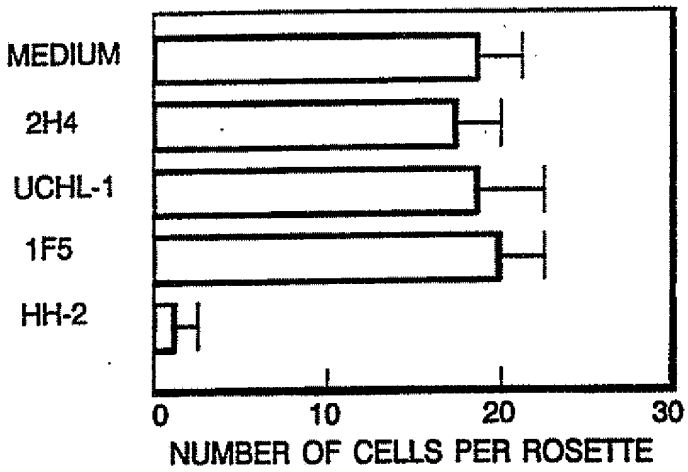

FIG. 9d

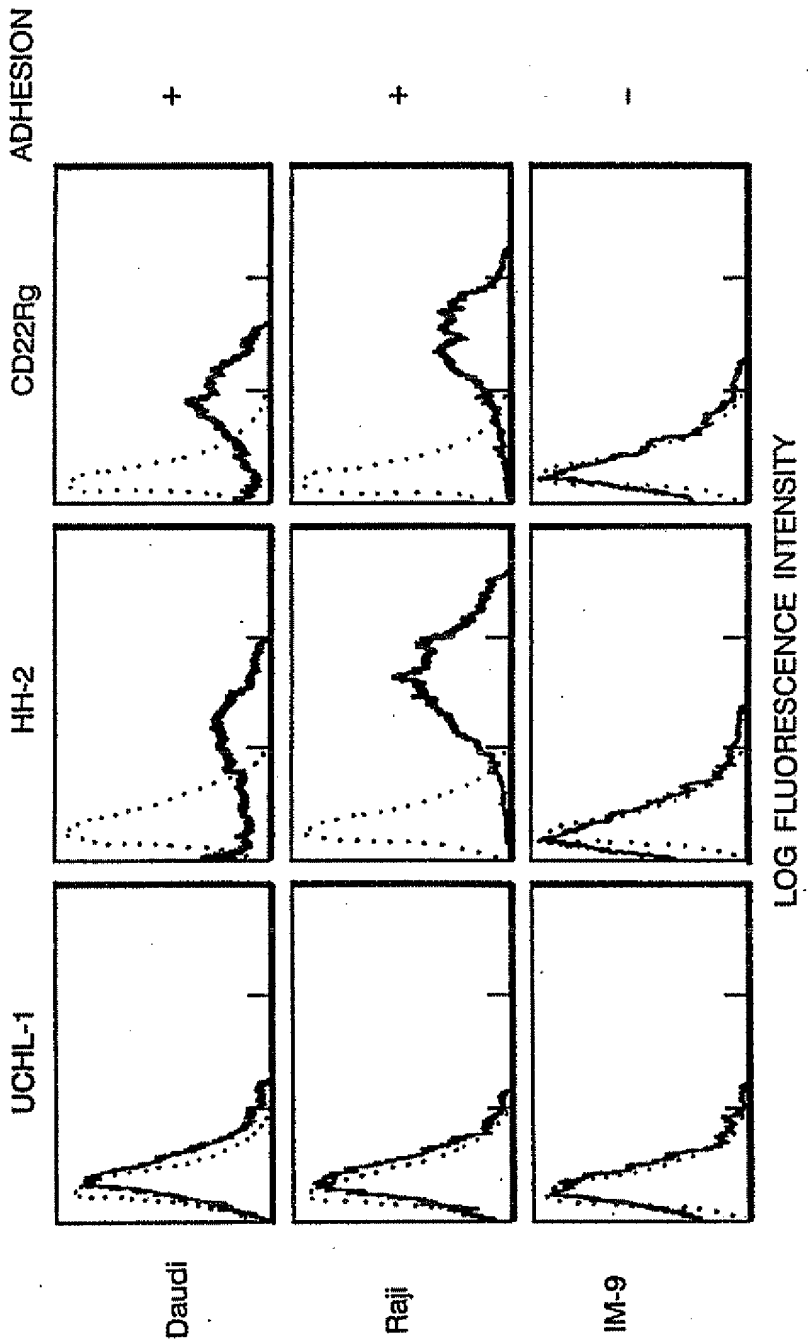
ADHESION
+
+
-
CD22Rg
HH-2
UCHL-1
Daudi
Raji
IM-9
LOG FLUORESCENCE INTENSITY

FIG. 9c

```
CCATCCCATA GTGAGGGAAG ACACGCGGAA ACAGGCTTGC ACCCAGACAC GACACC

ATG CAT CTC CTC GGC CCC TGG CTC CTG CTC CTG GTT CTA GAA TAC TTG
Met His Leu Leu Gly Pro Trp Leu Leu Leu Leu Val Leu Glu Tyr Leu
1               5                  10                  15

GCT TTC TCT GAC TCA AGT AAA TGG GTT TTT GAG CAC CCT GAA ACC CTC
Ala Phe Ser Asp Ser Ser Lys Trp Val Phe Glu His Pro Glu Thr Leu
            20                  25                  30

TAC GCC TGG GAG GGG GCC TGC GTC TGG ATC CCC TGC ACC TAC AGA GCC
Tyr Ala Trp Glu Gly Ala Cys Val Trp Ile Pro Cys Thr Tyr Arg Ala
        35                  40                  45

CTA GAT GGT GAC CTG GAA AGC TTC ATC CTG TTC CAC AAT CCT GAG TAT
Leu Asp Gly Asp Leu Glu Ser Phe Ile Leu Phe His Asn Pro Glu Tyr
    50                  55                  60

AAC AAG AAC ACC TCG AAG TTT GAT GGG ACA AGA CTC TAT GAA AGC ACA
Asn Lys Asn Thr Ser Lys Phe Asp Gly Thr Arg Leu Tyr Glu Ser Thr
65                  70                  75                  80

AAG GAT GGG AAG GTT CCT TCT GAG CAG AAA AGG GTG CAA TTC CTG GGA
Lys Asp Gly Lys Val Pro Ser Glu Gln Lys Arg Val Gln Phe Leu Gly
                85                  90                  95

GAC AAG AAT AAG AAC TGC ACA CTG AGT ATC CAC CCG GTG CAC CTC AAT
Asp Lys Asn Lys Asn Cys Thr Leu Ser Ile His Pro Val His Leu Asn
            100                 105                 110

GAC AGT GGT CAG CTG GGG CTG AGG ATG GAG TCC AAG ACT GAG AAA TGG
Asp Ser Gly Gln Leu Gly Leu Arg Met Glu Ser Lys Thr Glu Lys Trp
        115                 120                 125

ATG GAA CGA ATA CAC CTC AAT GTC TCT GAA AGG CCT TTT CCA CCT CAT
Met Glu Arg Ile His Leu Asn Val Ser Glu Arg Pro Phe Pro Pro His
    130                 135                 140

ATC CAG CTC CCT CCA GAA ATT CAA GAG TCC CAG GAA GTC ACT CTG ACC
Ile Gln Leu Pro Pro Glu Ile Gln Glu Ser Gln Glu Val Thr Leu Thr
145                 150                 155                 160

TGC TTG CTG AAT TTC TCC TGC TAT GGG TAT CCG ATC CAA TTG CAG TGG
Cys Leu Leu Asn Phe Ser Cys Tyr Gly Tyr Pro Ile Gln Leu Gln Trp
                165                 170                 175

CTC CTA GAG GGG GTT CCA ATG AGG CAG GCT GCT GTC ACC TCG ACC TCC
Leu Leu Glu Gly Val Pro Met Arg Gln Ala Ala Val Thr Ser Thr Ser
            180                 185                 190

TTG ACC ATC AAG TCT GTC TTC ACC CGG AGC GAG CTC AAG TTC TCC CCA
Leu Thr Ile Lys Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro
        195                 200                 205
```

FIG. 10a

```
CAG TGG AGT CAC CAT GGG AAG ATT GTG ACC TGC CAG CTT CAG GAT GCA
Gln Trp Ser His His Gly Lys Ile Val Thr Cys Gln Leu Gln Asp Ala
    210                 215                 220

GAT GGG AAG TTC CTC TCC AAT GAC ACG GTG CAG CTG AAC GTG AAG CAC
Asp Gly Lys Phe Leu Ser Asn Asp Thr Val Gln Leu Asn Val Lys His
225                 230                 235                 240

ACC CCG AAG TTG GAG ATC AAG GTC ACT CCC AGT GAT GCC ATA GTG AGG
Thr Pro Lys Leu Glu Ile Lys Val Thr Pro Ser Asp Ala Ile Val Arg
                245                 250                 255

GAG GGG GAC TCT GTG ACC ATG ACC TGC GAG GTC AGC AGC AGC AAC CCG
Glu Gly Asp Ser Val Thr Met Thr Cys Glu Val Ser Ser Ser Asn Pro
            260                 265                 270

GAG TAC ACG ACG GTA TCC TGG CTC AAG GAT GGG ACC TCG CTG AAG AAG
Glu Tyr Thr Thr Val Ser Trp Leu Lys Asp Gly Thr Ser Leu Lys Lys
        275                 280                 285

CAG AAT ACA TTC ACG CTA AAC CTG CGC GAA GTG ACC AAG GAC CAG AGT
Gln Asn Thr Phe Thr Leu Asn Leu Arg Glu Val Thr Lys Asp Gln Ser
    290                 295                 300

GGG AAG TAC TGC TGT CAG GTC TCC AAT GAC GTG GGC CCG GGA AGG TCG
Gly Lys Tyr Cys Cys Gln Val Ser Asn Asp Val Gly Pro Gly Arg Ser
305                 310                 315                 320

GAA GAA GTG TTC CTG CAA GTG CAG TAT GCC CCG GAA CCT TCC ACG GTT
Glu Glu Val Phe Leu Gln Val Gln Tyr Ala Pro Glu Pro Ser Thr Val
                325                 330                 335

CAG ATC CTC CAC TCA CCG GCT GTG GAG GGA AGT CAA GTC GAG TTT CTT
Gln Ile Leu His Ser Pro Ala Val Glu Gly Ser Gln Val Glu Phe Leu
            340                 345                 350

TGC ATG TCA CTG GCC AAT CCT CTT CCA ACA AAT TAC ACG TGG TAC CAC
Cys Met Ser Leu Ala Asn Pro Leu Pro Thr Asn Tyr Thr Trp Tyr His
        355                 360                 365

AAT GGG AAA GAA ATG CAG GGA AGG ACA GAG GAG AAA GTC CAC ATC CCA
Asn Gly Lys Glu Met Gln Gly Arg Thr Glu Glu Lys Val His Ile Pro
    370                 375                 380

AAG ATC CTC CCC TGG CAC GCT GGG ACT TAT TCC TGT GTG GCA GAA AAC
Lys Ile Leu Pro Trp His Ala Gly Thr Tyr Ser Cys Val Ala Glu Asn
385                 390                 395                 400

ATT CTT GGT ACT GGA CAG AGG GGC CCG GGA GCT GAG CTG GAT GTC CAG
Ile Leu Gly Thr Gly Gln Arg Gly Pro Gly Ala Glu Leu Asp Val Gln
                405                 410                 415
```

FIG. 10b

```
TAT CCT CCC AAG AAG GTG ACC ACA GTG ATT CAA AAC CCC ATG CCG ATT
Tyr Pro Pro Lys Lys Val Thr Thr Val Ile Gln Asn Pro Met Pro Ile
            420                 425                 430

CGA GAA GGA GAC ACA GTG ACC CTT TCC TGT AAC TAC AAT TCC AGT AAC
Arg Glu Gly Asp Thr Val Thr Leu Ser Cys Asn Tyr Asn Ser Ser Asn
        435                 440                 445

CCC AGT GTT ACC CGG TAT GAA TGG AAA CCC CAT GGC GCC TGG GAG GAG
Pro Ser Val Thr Arg Tyr Glu Trp Lys Pro His Gly Ala Trp Glu Glu
    450                 455                 460

CCA TCG CTT GGG GTG CTG AAG ATC CAA AAC GTT GGC TGG GAC AAC ACA
Pro Ser Leu Gly Val Leu Lys Ile Gln Asn Val Gly Trp Asp Asn Thr
465                 470                 475                 480

ACC ATC GCC TGC GCA CGT TGT AAT AGT TGG TGC TCG TGG GCC TCC CCT
Thr Ile Ala Cys Ala Arg Cys Asn Ser Trp Cys Ser Trp Ala Ser Pro
                485                 490                 495

GTC GCC CTG AAT GTC CAG TAT GCC CCC CGA GAC GTG AGG GTC CGG AAA
Val Ala Leu Asn Val Gln Tyr Ala Pro Arg Asp Val Arg Val Arg Lys
            500                 505                 510

ATC AAG CCC CTT TCC GAG ATT CAC TCT GGA AAC TCG GTC AGC CTC CAA
Ile Lys Pro Leu Ser Glu Ile His Ser Gly Asn Ser Val Ser Leu Gln
        515                 520                 525

TGT GAC TTC TCA AGC AGC CAC CCC AAA GAA GTC CAG TTC TTC TGG GAG
Cys Asp Phe Ser Ser Ser His Pro Lys Glu Val Gln Phe Phe Trp Glu
    530                 535                 540

AAA AAT GGC AGG CTT CTG GGG AAA GAA AGC CAG CTG AAT TTT GAC TCC
Lys Asn Gly Arg Leu Leu Gly Lys Glu Ser Gln Leu Asn Phe Asp Ser
545                 550                 555                 560

ATC TCC CCA GAA GAT GCT GGG AGT TAC AGC TGC TGG GTG AAC AAC TCC
Ile Ser Pro Glu Asp Ala Gly Ser Tyr Ser Cys Trp Val Asn Asn Ser
                565                 570                 575

ATA GGA CAG ACA GCG TCC AAG GCC TGG ACA CTT GAA GTG CTG TAT GCA
Ile Gly Gln Thr Ala Ser Lys Ala Trp Thr Leu Glu Val Leu Tyr Ala
            580                 585                 590

CCC AGG AGG CTG CGT GTG TCC ATG AGC CCG GGG GAC CAA GTG ATG GAG
Pro Arg Arg Leu Arg Val Ser Met Ser Pro Gly Asp Gln Val Met Glu
        595                 600                 605

GGG AAG AGT GCA ACC CTG ACC TGT GAG AGT GAC GCC AAC CCT CCC GTC
Gly Lys Ser Ala Thr Leu Thr Cys Glu Ser Asp Ala Asn Pro Pro Val
    610                 615                 620
```

FIG. 10c

SUBSTITUTE SHEET

```
TCC CAC TAC ACC TGG TTT GAC TGG AAT AAC CAA AGC CTC CCC CAC CAC
Ser His Tyr Thr Trp Phe Asp Trp Asn Asn Gln Ser Leu Pro His His
625                 630                 635                 640

AGC CAG AAG CTG AGA TTG GAG CCG GTG AAG GTC CAG CAC TCG GGT GCC
Ser Gln Lys Leu Arg Leu Glu Pro Val Lys Val Gln His Ser Gly Ala
                645                 650                 655

TAC TGG TGC CAG GGG ACC AAC AGT GTG GGC AAG GGC CGT TCG CCT CTC
Tyr Trp Cys Gln Gly Thr Asn Ser Val Gly Lys Gly Arg Ser Pro Leu
            660                 665                 670

AGC ACC CTT ACT GTC TAC TAT AGC CCG GAG ACC ATC GGC AGG CGA GTG
Ser Thr Leu Thr Val Tyr Tyr Ser Pro Glu Thr Ile Gly Arg Arg Val
        675                 680                 685

GCT GTG GGA CTC GGG TCC TGC CTC GCC ATC CTC ATC CTG GCA ATC TGT
Ala Val Gly Leu Gly Ser Cys Leu Ala Ile Leu Ile Leu Ala Ile Cys
    690                 695                 700

GGG CTC AAG CTC CAG CGA CGT TGG AAG AGG ACA CAG AGC CAG CAG GGG
Gly Leu Lys Leu Gln Arg Arg Trp Lys Arg Thr Gln Ser Gln Gln Gly
705                 710                 715                 720

CTT CAG GAG AAT TCC AGC GGC CAG AGC TTC TTT GTG AGG AAT AAA AAG
Leu Gln Glu Asn Ser Ser Gly Gln Ser Phe Phe Val Arg Asn Lys Lys
                725                 730                 735

GTT AGA AGG GCC CCC CTC TCT GAA GGC CCC CAC TCC CTG GGA TGC TAC
Val Arg Arg Ala Pro Leu Ser Glu Gly Pro His Ser Leu Gly Cys Tyr
            740                 745                 750

AAT CCA ATG ATG GAA GAT GGC ATT AGC TAC ACC ACC CTG CGC TTT CCC
Asn Pro Met Met Glu Asp Gly Ile Ser Tyr Thr Thr Leu Arg Phe Pro
        755                 760                 765

GAG ATG AAC ATA CCA CGA ACT GGA GAT GCA GAG TCC TCA GAG ATG CAG
Glu Met Asn Ile Pro Arg Thr Gly Asp Ala Glu Ser Ser Glu Ile Gln
    770                 775                 780

AGA CCT CCC CGG ACC TGC GAT GAC ACG GTC ACT TAT TCA GCA TTG CAC
Arg Pro Pro Arg Thr Ser Asp Asp Thr Val Thr Tyr Ser Ala Leu His
785                 790                 795                 800

AAG CGC CAA GTG GGC GAC TAT GAG AAC GTC ATT CCA GAT TTT CCA GAA
Lys Arg Gln Val Gly Asp Tyr Glu Asn Val Ile Pro Asp Phe Pro Glu
                805                 810                 815

GAT GAG GGG ATT CAT TAC TCA GAG CTG ATC CAG TTT GGG GTC GGG GAG
Asp Glu Gly Ile His Tyr Ser Glu Leu Ile Gln Phe Gly Val Gly Glu
            820                 825                 830
```

FIG. 10d

```
CGG CCT CAG GCA CAA GAA AAT GTG GAC TAT GTG ATC CTC AAA CAT TGA
Arg Pro Gln Ala Gln Glu Asn Val Asp Tyr Val Ile Leu Lys His
      835                 840                 845

CACTGGATGG GCTGCAGCAG AGGCACTGGG GGCAGCGGGG GCCAGGGAAG TCCCCGAGTT
TCCCCAGACA CCGCCACATG GCTTCCTCCT GCGTGCATGT GCGCACACAC ACACACACAC
GCACACACAC ACACACACAC TCACTGCGGA GAACCTTGTG CCTGGCTCAG AGCCAGTCTT
TTTGGTGAGG GTAACCCCAA ACCTCCAAAA CTCCTGCCCC TGTTCTCTTC CACTCTCCTT
GCTACCCAGA AATCATCTAA ATACCTGCCC TGACATGCAC ACCTCCCCTG CCCCACCAGC
CCACTGGCCA TCTCCACCCG GAGCTGCTGT GTCCTCTGGA TCTGCTCGTC ATTTTCCTTC
CCTTCTCCAT CTCTCTGGCC CTCTACCCCT GATCTGACAT CCCCACTCAG GAATATTATG
CCCAGTTTCT GCCTCTGAGG AAAAGGACAG AAACGAAGTA GAAAGGGGCC CAGTCCTGGC
CTGGCTTCTC CTTTGGAAGT GAGGCATTGC ACGGGGAGAC GTACGTATCA GCGGCCCCTT
GACTCTGGGG ACTCCGGGTT TGAGATGGAC ACACTGGTGT GGATTAACCT GCCAGGGAGA
CAGAGCTCAC AATAAAAATG GCTCAGATGC CACTTCAAAG AAAAAAAAA
```

FIG. 10e

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/07994

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 45/05, 37/00; C12N 15/19, 15/24, 15/09; C07K 13/00
US CL :424/85, 85.2; 435/7.24, 41, 69.6, 320.1; 514/2; 530/351
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85, 85.2; 435/7.24, 41, 69.6, 320.1; 514/2; 530/351

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Biosis, Medline, Chem Ab, Derwent WPI, APS (CD22, gene, cloning, BL-Cam, Leu-14, T cells, CD22 beta, B cells, authors names

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FASEB Journal, Volume 4, issued 26 April 1990, G.L. Wilson et al., "Identification of a probable B Lymphocyte homotypic cell adhesion molecule, BL-CAM", page A1698, NO. 23.	1-13
X	Journal of Experimental Medicine, volume 173, issued January 1991, G.L. Wilson et al., "cDNA Cloning CD22: A Mediator of B-B cell Interactions:, pages 137-146, see entire document.	1-13
A	Journal of Immunology, Volume 140, issued 01 January 1988, D.R. Boue et al., "Structural Characterization of the Human B Lymphocyte Restricted Differentiation Antigen CD22" pages 192-199, see entire document.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 DECEMBER 1992	Date of mailing of the international search report 16 DEC 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer RON SCHWADRON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*